ASSESSMENT OF FACTORS AFFECTING ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

OTOLOG KÖK HÜCRE TRANSPLANTASYONU SONRASI ENGRAFMANI ETKİLEYEN FAKTÖRLERİN DEĞERLENDİRİLMESİ

ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

Fatma Dogruel[1], Cigdem Pala Ozturk[2], Murat Albayrak[2], Leylagul Kaynar[3], Serdar Sıvgın[3], Bulent Eser[3], Ali Unal[3], Mustafa Cetin[3]
[1]Department of Internal Medicine, Erciyes University, Faculty of Dentistry, Kayseri,
[2]Diskapi Yildirim and Beyazit Training Research Hospital, Ankara,
[3]Department of Hematology, Erciyes University, Faculty of Medicine, Kayseri, Türkiye

Öz

Amaç: Bu çalışmada miyeloablasyondan sonra periferik kök hücrelerin infüzyonunu takiben engrafman gelişimini etkileyebilecek faktörleri belirlemek amaçlanmıştır. Gereç ve Yöntem: Otologhematopoetik kök hücre nakli (HKHN) yapılan 121 hastanın verileri retrospektif olarak değerlendirildi.Hastaların 39'u (%32) MultipleMyelom (MM), 34'ü (%28) Nonhodgkin Lenfoma (NHL), 33'ü (%27) hodgkin lenfoma (HL), 9'u (%8) akut lösemi, 6'sı (%5) solid tümörlü hasta idi. Hazırlama rejimi olarak BEAM (karmustin, etoposid, arabinozid-C, melfalan), yüksek doz ICE (ifosfamid, karboplatin, etoposid), ME (melfalan+etoposid), BuCy (busulfan+siklofosfamid) ve melfalankullanıldı. Hastaların yaş ortalaması 41.3 olup ortanca yaş 43 idi (16-71 yıl). Kök hücre infüzyonunutakibenengrafman gelişmesinde ilgili olabilecek faktörleri belirlemek için nakil sırasında kullanılan hazırlama rejimi, infüzyondan sonra uygulanan büyüme faktör tipi, infüze edilen toplam CD34+ hücre sayısı, nakilden sonra nötropenik ateş oluşumu değerlendirildi. Bulgular: Nötrofil (500/µl) ve trombosit (20000/µl) tutunma sayıları sırasıyla ortalama 10,1±2,3 ve 11,5±3,9 günde ulaşıldı. Hastaların tanı, cinsiyet, nakil öncesi alınan RT ve kullanılan büyüme faktörünün engrafman zamanı üzerine anlamlı etkisi bulunmadı (p>0,05). CD34+ hücre sayısı (dozu) kademeli olarak arttıkça engrafmanın daha hızlı olduğu gözlendi (p<0,05). Hazırlama rejimlerinden ICE kullanılan hastalarda BEAM kullanılan hastalara göre platelet engrafmanının daha hızlı olduğu ayrıca nötrofil engrafmanının ICE kullanılan hastalarda Melfalan kullananlara göre daha hızlı olduğu saptanmıştır (p<0,05). CD34 + hücre sayısı ve engrafman arasında doğrudan bir ilişki gösterilememişken, yüksek CD34 + hücre sayısında engraftmanın daha hızlı olduğu bulunmuştur. Tartışma: Kemik iliği rejenerasyonu arttırmak için optimum periferik kök hücre nakli protokolünü tanımlamak; miyeloablatif tedavi sonrası hematopoetik yenilenmeyi hızlandırmak ve engrafman üzerine etkileyen faktörleri belirlemek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

Otolog Hematopoetik Kök Hücre Nakli; Engrafman (Tutunma); Hazırlama Rejimi

Abstract

Aim: The aim of this study was to identify factors that may be related to the development of engraftment following peripheral stem cell infusion after myeloablation. Material and Method: The data of 121 patients who underwent autologous hematopoietic stem cell transplantation were retrospectively reviewed. Of the patients, 39 (32%) had multiple myeloma (MM), 34 (28%) had non-Hodgkin lymphoma (NHL), 33 (27%) had Hodgkin lymphoma (HL), 9 (8%) had acute leukemia and 6 (5%) had solid tumor.BEAM (carmustine, etoposide, arabinoside-C, melphalan), high doses of ICE (ifosfamide, carboplatin, etoposide), ME (melphalan plus etoposide), BuCy (busulfan and cyclophosphamide), and melphalan were used as preparation regimes. The mean age was 41.3 years with a median of 43 years (range 16-71 years). To identify factors that may be relevant in the development of engraftment following stem cell infusion, preparation regime, type of growth factor, total CD34+ cell count, and neutropenic fever occurrence were evaluated. Results: Neutrophil >500/µl and plt >20,000/µl levels were achieved at 10 ,1± 2 ,3 and11 ,5 ± 3 ,9 days, respectively. Patients age, gender, diagnosis, radiotherapy before transplantation, and G-CSF administration had no significant effect on engraftment time. It was found that platelet engraftment was more rapid in patients who underwent ICE than in those who underwent the BEAM preparation regime; in addition, it was found that neutrophil engraftment was more rapid in patients who underwent ICE than in those who underwent the melphalan preparation regime. No direct correlation was demonstrated between CD34+ cell count and engraftment, while the engraftment was found to be more rapid in higher CD34+ cell counts. Discussion: Further studies are needed to identify the optimal peripheral stem cell transplantation protocol to improve bone marrow regeneration; to accelerate hematopoietic regeneration after myeloablative therapy; and to determine factors influencing engraftment.

Keywords

Autologous Stem Cell Transplantation; Engraftment; Preparation Regime

DOI: 10.4328/JCAM.4813 Received: 26.09.2016 Accepted: 01.01.2017 Printed: 01.07.2017 J Clin Anal Med 2017;8(4): 332-5
Corresponding Author: Fatma Dogruel, Department of Internal Medicine, Erciyes University Faculty of Dentistry, Kayseri, Turkey.
T.: +90 3522076666-29183 F.: +90 3524380657 E-Mail: fdogruel@gmail.com

Introduction

Autologous stem cell transplantation describes the process whereby stem cells are previously obtained and given to the same patient after high-dose chemotherapy for the treatment of hematological diseases (multiple myeloma, recurrent lymphoma, acute myeloid leukemia) and solid organ tumors [1]. In autologous stem cell transplantation, one of the success criteria is the initiation and maintenance of blood element production for survival without the need for exogenous support by hematopoietic progenitor cells engrafting bone marrow. This process, which occurs within weeks after stem cell infusion, is termed engraftment. In engraftment kinetics, neutrophil engraftment is accepted as the first of 3 consecutive days in which the absolute neutrophil count reaches a level higher than 500 mm^3/L, while platelet engraftment is accepted as the first of 3 consecutive days in which the platelet count reaches a level higher than 20,000 mm^3/L without the need for platelet transfusion [2, 3].

It is important to predict the rate and long-term persistence of hematopoietic engraftment after autologous peripheral stem cell transplantation. This is because morbidity and mortality after transplantation, transfusion and antibiotic need, length of hospital stay, and resultant economic picture all depend on engraftment, which is an important factor for determining the success of transplantation. The neutrophil and platelet engraftment times can vary depending on many factors including underlying disease, age, radiotherapy before transplantation, and the G-CSF agent used in transplantation [4].

In the present study, our aim was to investigate factors affecting engraftment kinetics by retrospectively reviewing the charts and electronic database of 121 patients who underwent autologous peripheral stem cell transplantation between January, 2005 and January, 2010.

Material and Method

The present study was conducted by retrospectively reviewing the outcomes of 121 patients who underwent autologous stem cell transplantation at the Erciyes Transplantation Center in the Hematology Department of Erciyes University Medical School between January, 2005 and January, 2010. Approval from the local Ethics Committee was obtained prior to commencing the study and it was carried out according to the principles of the Helsinki Declaration.

The data were obtained from patient charts and the electronic database. All patients were informed about the early and late complications of transplantation; then, they gave informed consent before transplantation.

The diagnosis age, gender, radiotherapy status, preparation regime used in the transplantation procedure, CD34+ cell count given during transplantation, and G-CSF agent used after transplantation were recorded for all patients.

Preparation Regimes

The following regimes were used:

• Melphalan (n=39) (200 mg/m^2 -3. day),

• BEAM (n=55) [BCNU 300 mg/m^2 (-8. day), etoposide 200 mg/m^2 (-7, -6, -5, -4. days), cytosine arabinoside 2x100 mg/m^2 (-7, -6, -5, -4. days), and melphalan 140 mg/m^2 (-3. day)]

HD ICE (n=14) [ifosfamide 2,5 gr/m^2(-8, -7, -6, -5, -4, -3.days), carboplatin 250 mg/m^2(-8, -7, -6, -5, -4, -3.days), etoposide100 mg/m^2(-5, -4, -3. days)]

• Cyclophosphamide plus busulfan (n=9) [busulfan 3,2 mg/kg/day (-7, -6, -5, -4. days), cyclophosphamide 60 mg/kg/day (-3, 2. days), mesna 120 mg/kg/day (-3, -2,-1. days)]

• Melphalan plus etoposide (n=4) [melphalan 60 mg/m^2(-5, -4, -3. days), etoposide100 mg/m^2(-5, -4, -3. days)] [5].

Stem Cell Transplantation and G-CSF Administration

In the present study, peripheral stem cells were used as the stem cell sourcein all patients who underwent autologous stem cell transplantation. On day 1 after stem cell transplantation, the G-CSF agent (lenograstim, filgrastim) was initiated and continued until engraftment [6, 7].

Parameters Evaluated for Effects on Engraftment Time

In the post-transplantation period, neutrophil engraftment was accepted as the first of the 3 consecutive days in which the neutrophil count was higher than 500mm^3/L, while platelet engraftment was accepted as the first of the 3 consecutive days in which the platelet count was higher than 20,000 mm^3/L [2, 3]. After transplantation, the emergence of white blood cells and platelets was assessed within days. The effects of diagnosis, age, gender, radiotherapy status, preparation regime used in transplantation procedure, CD34+ cell count given during transplantation, and G-CSF agent used after transplantation on these parameters were evaluated.

Statistical Analysis

The patient charts and electronic database were used to obtain patient data. Mean values and standard deviation were calculated for the numeric values included. Mann-Whitney U and Kruskal-Wallis tests were used to determine factors affecting engraftment. $P<0.05$ was considered as significant. SPSS for Windows version 15.0 was used for statistical analysis.

Results

Of the patients who underwent autologous transplantation, 80 (66.1%) were male while 41 (33.9%) were female. The mean age was 41.3 years with a median of 43 years (range 16-71 years). Table 1 presents the gender, diagnoses, and distribution of preparation regimes.

Of the patients with acute leukemia, 8 had AML while one hadbiphenotypic leukemia. Of the patients with solid tumors, Ewing sarcoma was present in 2 patients and choriocarcinoma, osteosarcoma, PNET, and testis tumor in one patient each.

The mean amount of CD34+ hematopoietic stem cells given to patients was 5.98±3.98x10^6/kg with a median value of 5.2x10^6/kg (min:0.6x10^6/kg-max:19.57x10^6/kg). In patients who underwent transplantation, neutrophil engraftment developed in a mean time of 10.1±2.3 days with a median of 10 days (min:7-max:26 days) while platelet engraftment developed in a mean time of 11.5±3.9 days with a median of 11 days (min:6-max:33 days). Fifty (42%) patients received radiotherapy before the preparation phase.

Neutrophil engraftment developed in all patients after transplantation. However, platelet engraftment failed in one patient with Hodgkin lymphoma. Table 2 presents the leukocyte and platelet engraftment times according to diagnosis.

Patients were stratified into 4 groups to evaluate the effect on engraftment of CD34 + cell count given (dose) (Table 3). The effects of CD34+ cell count given on times of neutrophil, Neu 500, Neu 1500, and Neu 2500 engraftment and times of platelet, platelet 20,000, platelet 50,000, and platelet 100,000 engraftment were evaluated according to this stratification.

The effects of preparation regimes and G-CSF agents used during transplantation on engraftment were also evaluated. It was found that platelet engraftment was more rapid in patients who received the ICE preparation regime when compared to those who received the BEAM regime (p=0.021). Also, it was found that neutrophil engraftment was more rapid in patients who received the ICE preparation regime when compared to those who received melphalan (p=0.002).No significant difference was detected between the other regimes. It was found that diagnosis, gender, neutropenic fever, and radiotherapy and growth factors used had no significant effect on engraftment.

Table 1. Distribution of gender, diagnosis, and preparation regimes in patients who underwent autologous peripheral stem cell transplantation.

		Number of patients (n)
Gender	Female	41 (33.9 %)
	Male	80 (66.1 %)
Diagnosis		
	MM	39 (32 %)
	NHL	34 (28 %)
	HL	33 (27 %)
AcuteLeukemia		9 (8 %)
Solid Tumor		6 (5 %)
Preparation Regime		
	BEAM Regime	55 (46 %)
Melphalan		39 (32 %)
	HD ICE	14 (12 %)
Busulfan+Cyc.		9 (7 %)
Melphalan+Etoposide		4 (3 %)

MM:MultipleMyeloma, NHL:NonHodgkinLymphoma, HL:Hodgkinlymphoma, BEAM: BCNU (Carmustine)+Etoposide+Melphalan+CytosineArabin oside, HD ICE (High dose ICE): Ifosfamide+carboplatin+Etoposide, Cyc:Cyclophosphamide.

Table 3. Stratification according to CD 34+ cell counts

Groups	CD 34+ (10^6 /kg)	Number of patients (n)
A	0.6-2.5	13
B	2.5-4.9	38
C	4.9-7.8	32
D	>7.8	30

Discussion

Autologous stem cell transplantation and high-dose chemotherapy are effective in the majority of malign diseases. This approach is increasingly used in the treatment of hematological malignancies and selected solid tumors. Our findings showed that the preparation regime used before transplantation had an effect on engraftment as a factor in autologous stem cell transplantation. However, according to a previous study, there were no significant differences in terms of engraftment between the BEAM and ICE conditioning regimens [8].

Although the mechanism underlying hematopoietic regeneration has not been fully elucidated, it is widely accepted that bone marrow engraftment has several phases. As hematopoietic growth factors increase the number of progenitor cells at the periphery, the initial phase of engraftment is rapid in peripheral stem cell transplantation, in which growth factors are used for mobilization [9,10].

The engraftment time for leukocyte and platelets can vary based on many factors. In EBMT and many studies, a neutrophil count>500 mm^3/L is accepted as a threshold for leukocyte engraftment, while a platelet count>20,000 mm^3/L is accepted as a threshold for platelet engraftment.

The threshold CD34+ cell count should be 2, 0–2, 5x10^6/kg for timely hematological recovery [11, 12] and CD34+ cell counts should be higher than 10x10^6/kg [13] and 15x10^6/kg [14] to accelerate engraftment. It is known that there is a positive correlation between engraftment kinetics and CD34+ cell count. In our study, no significant difference was observed whenthe CD34+ cell count was considered in a direct evaluation. However, it was found that engraftment was more rapid in the group with a CD34+ cell count>7.5x10^6/kg when patients were evaluated by stratifying into 4 groups according to CD34+ cell count. It was thought that lack of significant effect of the CD34+ cell count on either leukocyte or platelet engraftment could be explained by the infusion of apheresis product which contains redundant amounts of CD34+ cells (5.98±3.98x10^6/kg).

Only one patient received CD34+ cells of 0.6x10^6/kg and engraftment developed on the expected days (leukocyte engraftment: day 11 and platelet engraftment: day 12). In the group with the lowest number of CD34+ cells, leukocyte and platelet engraftment developed on day 10.4 and 9.9, respectively. When compared to the groups with higher numbers of CD34+ cells, no significant difference was detected regarding engraftment time. Although blood values increased more rapidly with a higher number of CD34 cells after engraftment, particularly at levels above 7.8x10^6/kg, use of lower levels should not be avoided in transplantation.

G-CSF promotes neutrophil production in the bone marrow and its functions. The unglycosylated form of G-CSF is called fil-

Table 2. Neutrophil and platelet engraftment times according to diagnosis

	Plt 20.000 mm^3/L (day)	Plt 50,000 mm^3/L (day)	Plt 100,000 mm^3/L (day)	Neu mm^3/L (day)	Neu 1500 mm^3/L (day)	Neu 2500 mm^3/L (day)
MM	11.4±2.9	17.9±12.9	24.6±11	10.5±2.1	10.6±1.9	11.3±2.3
NHL	12.1±5.1	21.8±14.2	25.6±13.4	9.5±1.6	10.1±2.2	10.3±1.7
HL	11±3.8	11.1±4.3	19.2±8.4	10.1±3.3	10.6±3.7	10.5±2.1
Acute Leukemia	12±3.2	48±60.4	97±116.9	10.6±1.8	11.4±2.3	12.2±2.6
Solid Tumor	11.8±3.1	14.3±5.8		9.5±1.3	9.8±1.6	10.3±1.5

Plt:Platelet, Neu:Neutrophil, MM:MultipleMyeloma, NHL:NonHodgkinLymphoma, HL:Hodgkinlymphoma,

grastim while the glycosylated form is called lenograstim.

In our study, no significant difference was detected between G-CSF agents, when lenograstim and filgrastim were compared for their effects on engraftment (leukocyte p=0.787, platelet p=0.253). As no definitive differences have been established regarding the growth factors used after transplantation, clinicians should take other factors such as cost and feasibility into consideration when selecting the growth factor.

Given the possibility that there may be some changes in the reserves of bone marrow with age, we evaluated the effects of age on hematopoietic engraftment time. In our study, no significant relationship was found between age and leukocyte (p=0.127) and platelet engraftment times (p=0.492), when patients who underwent peripheral stem cell transplantation were stratified according to age. Given the finding that there was no significant difference (platelet engraftment p=0.130, leukocyte engraftment p=0.057) even between patients younger than 20 and older than 60 years of age, it may be suggested that age alone is not a limiting factor regarding transplantation.

Some authors reported that there might be a delay in the regeneration of bone marrow in cases who received radiotherapy before the preparation phase for peripheral stem cell transplantation [15]. Accordingly, it has been advocated that apheresis should be performed more intensively in cases scheduled for the peripheral stem cell transplantation program after radiotherapy to a large area, particularly tothe pelvic region [9, 10]. In our cases, the engraftment times were not significantly different in cases who received no radiotherapy. Given the fact that hematopoietic regeneration is delayed in cases who received intensive chemotherapy, it may be suggested that radiotherapy-based treatments should be preferably considered in cases in which transplantation is planned.

In our study, it may be concluded that radiotherapy-based treatments should be primarily considered in cases in which transplantation is planned, and also that age is not a limiting factor regarding engraftment based on the findings that radiotherapy and age had no significant effect on engraftment. Further studies are needed to determine optimal stem cell transplantation protocol; to accelerate hematopoietic regeneration after myeloablative therapy; and to identify factors that affect engraftment.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

The authors declare that they have no competing interests.

References

1. Armitage JO. The History of Autologous Hematopoetic Cell Transplantation, In : Appelbaum FR, Forman SJ, Negrin KG, editors. Thomas' Hematopoietic Cell Transplantation. United Kingdom: Wiley-Blackwell; 2009. p.8-14.
2. Davies SM, Ramsay NK, Haake RJ, Kersey JH, Weisdorf DJ, McGlave PB, et al. Comparison of engraftment in recipients of matched sibling of unrelated donor marrow allografts. Bone Marrow Transplant 1994;13:51-7.
3. Derenzini E, Stefoni V, Maglie R Casadei B, Pellegrini C, Broccoli A, et al. Collection of hematopoietic stem cells after previous radio immunotherapy is feasible and does not impair engraftment after autologous stem cell transplantation in follicular lymphoma. Biol Blood Marrow Transplant 2013;19:1695-701.
4. Goncalves TL, Benvegnu DM, Bonfanti G. Specific Factors İnfluence the Success of Autologous and Allogeneic Hematopoietic Stem Cell Transplantation. Oxid Med Cell 2009;2:82-7.
5. Bensinger WI. High-dose Preparatory Regimens, In: Appelbaum FR, Forman SJ, Negrin KG, editors. Thomas' Hematopoietic Cell Transplantation. United Kingdom: Wiley-Blackwell; 2009.p.316-32.
6. Klumpp TR, Mangan KF, Goldberg SL, Pearlman ES, Macdonald JS. Granulocyte Colony-stimulating Factor Accelerates Neutrophil Engraftment Following Peripheral-blood Stem-cell Transplantations. J Clin Oncol 1995;13:1323-7.
7. Piccirillo N, Sica S, Laurenti L, Chiusolo P, La Barbera EO, Sorà F, et al. Optimal Timing of G-CSF Administration after CD34+Immunoselected Peripheral Blood Progenitor Cell Transplantation. Bone MarrowTransplant 1999;23:1245-50.
8. Esbah O, Tekgündüz E, Sirinoğlu Demiriz I, CivrizBozdağ S, Kaya A, Tetik A, et al. Finding The Optimal Conditioning Regimen For Relapsed/Refractory Lymphoma Patients Undergoing Autologous Hematopoietic Cell Transplantation: A Retrospective Comparison Of Beamand High Dose ICE. Turk J Haematol 2015; doi: 10.4274/tjh.2014.0214.
9. Morse EE, Tuck D, Ascensao J, Sorba S, Dainiak N. Factors Affecting Recovery After Blood Stem Cell Transplantation. Ann Clin Lab Sci1993;23:89-96.
10. Bolwell BJ, Fishleder A, Andresen SW, Lichtin AE, Koo A, Yanssens T, et al. G-CSF Primed Peripheral Blood Progenitor Cells in Autologous Bone Marrow Transplantation: Parameters Affecting Bone Marrow Engraftment. Bone Marrow Transplantation 1993;12:609-14.
11. Chao NJ, Schriber JR, Grimes K, Long GD, Negrin RS, Raimondi CM, et al. Granulocyte colony-stimulating factor "mobilized" peripheral blood progenitor cells accelerate granulocyte and platelet recovery after high-dose chemotherapy. Blood 1993;81:2031-5.
12. To LB, Haylock DN, Simmons PJ, JuttnerCA. The Biology and Clinical Uses of Blood Stem Cells. Blood 1997;89:2233–58.
13. Weaver CH, Hazelton B, Birch R PalmerP,Allen C, Schwartzberg L, et al.An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood 1995;86:3961–9.
14. Ketterer N, Salles G, Raba M, Espinouse D, Sonet A, Tremisi P, et al. High CD34 cell counts decrease hematologic toxicity of autologous peripheral blood progenitor cell transplantation. Blood 1998;91:3148–55.
15. Tricot G, Jagannath S, Vesole D, Nelson J, Tindle S, Miller L, et al. Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. Blood 1995;85:588-96.

How to cite this article:

Dogruel F, Ozturk CP, Albayrak M, Kaynar L, Sıvgın S, Eser B, Unal A, Cetin M. Assessment of Factors Affecting Engraftment After Autologous Stem Cell Transplantation. J Clin Anal Med 2017;8(4): 332-5.